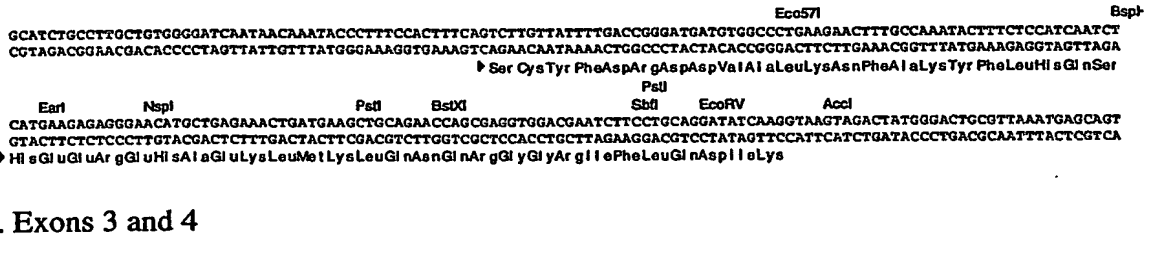
A. Exon 1

B. Exon 2



C. Exons 3 and 4

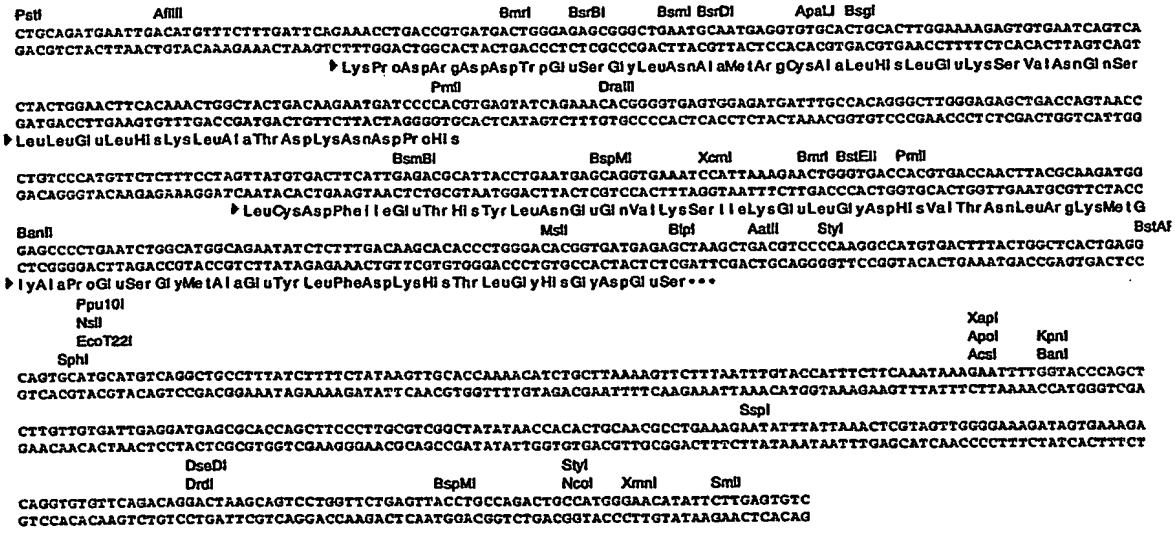


FIGURE 1

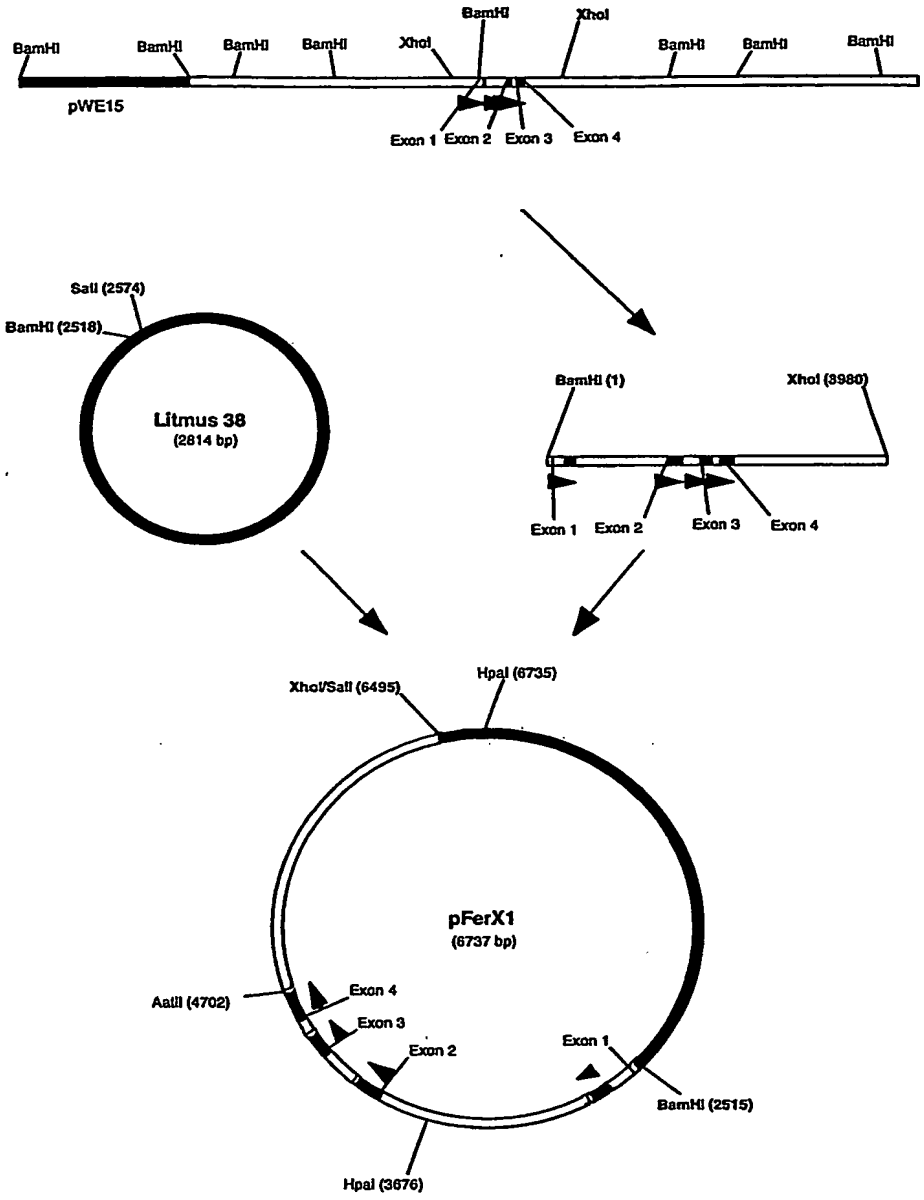

BamHI
BamHI
BamHI
BamHI
XhoI
BamHI
XhoI
BamHI
BamHI
BamHI
pWE15
Exon 1
Exon 2
Exon 3
Exon 4
SalI (2574)
BamHI (2518)
Litmus 38
(2814 bp)
BamHI (1)
XhoI (3980)
Exon 1
Exon 2
Exon 3
Exon 4
XhoI/SalI (6495)
HpaI (6735)
pFerX1
(6737 bp)
AatII (4702)
Exon 4
Exon 3
Exon 2
Exon 1
BamHI (2515)
HpaI (3676)

FIGURE 2

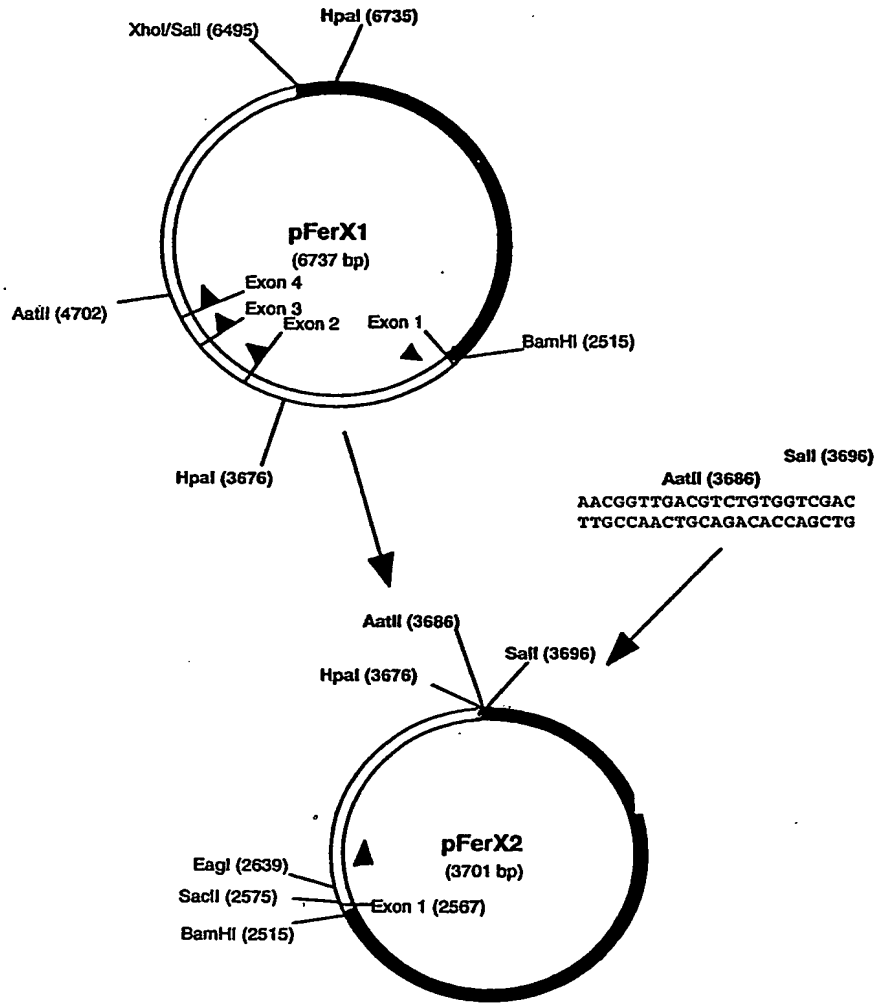

XhoI/SalI (6495)
HpaI (6735)
pFerX1
(6737 bp)
Exon 4
Exon 3
Exon 2
Exon 1
AatII (4702)
BamHI (2515)
HpaI (3676)
SalI (3696)
AatII (3686)
AACGGTTGACGTCTGTGGTCGAC
TTGCCAACTGCAGACACCAGCTG
AatII (3686)
SalI (3696)
HpaI (3676)
pFerX2
(3701 bp)
EagI (2639)
SacII (2575)
Exon 1 (2567)
BamHI (2515)

FIGURE 3

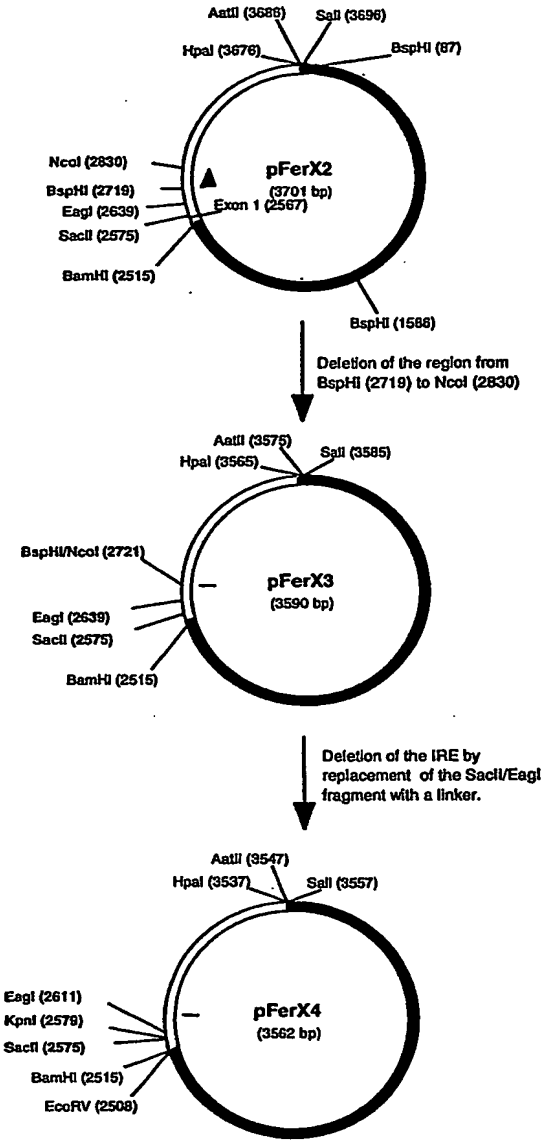

AatII (3686)
SalI (3696)
HpaI (3676)
BspHI (87)
NcoI (2830)
BspHI (2719)
EagI (2639)
SacII (2575)
BamHI (2515)
pFerX2
(3701 bp)
Exon 1 (2567)
BspHI (1586)
Deletion of the region from BspHI (2719) to NcoI (2830)
AatII (3575)
HpaI (3565)
SalI (3585)
BspHI/NcoI (2721)
EagI (2639)
SacII (2575)
BamHI (2515)
pFerX3
(3590 bp)
Deletion of the IRE by replacement of the SacII/EagI fragment with a linker.
AatII (3547)
HpaI (3537)
SalI (3557)
EagI (2611)
KpnI (2578)
SacII (2575)
BamHI (2515)
EcoRV (2508)
pFerX4
(3562 bp)

FIGURE 4

A.

Fer1

ATC TGT CCA TG GTGAGTGCGGCCTGGCCTTTGGCGGGGCGGAAAGAGGGTGCGGCCTGGCCTCCCTTGGGCCACTTGGTGAGCTGGCGGAGG

GTGGGTTGGGGCGTGGCCTGCTGCGGGCTTCCCCGCCTTCCAGCGCCCTTCTGGAAAATGGAGTTTGTCCGGGGTTCTTTCCAAAGGCAGGCAGCC
CTGCCGTGGCAAGTCTGAGCACCTAGCGCTTTGTGGCTCCTGCATAGACCAGGCACGTCATAACACCCGTGTTTTGAAGCCTTAGGGCTGTACAAC
TGTCAGCCTCTCCAATCAACCCTGCAGTTAGGTGCATTTTCCTGCACTCTCGTCCCCTCCGGTCACATGGCCTGCAGGCTTCTCTGTTTGGGTGTA
CATCCAGCTCCAGTTCCTCTGACTATGGCGGGTCTGCTTGGTCATGGTGTGGAATGGCAGCCCTGGGGCTTGGTACAAAGAGGCTTATCTCTTGTG
AACTTACTCTAACCACTTCTGAAGCAGCGGCCTCTACATCTCTGCTTATCACAGAGCCTCACTTGCATTGAAACTTATCGCTAGGAATCTCCCCTT
CTGTAATCACCCTGACCTTGCCAAGGCATCTAGAGTACTGTACGTTTTTAATTTTTATTTTGCACCAGTTGTTGCTTACTAACAGAAGTAGTAGGT
AACATACTTGTTGGAAAAAGCCCACGGTTGGGAAAAAACCATTATCGTGGAATACAAATACACTGAGTGCCTAAAACTGAAAATCAAAGCTTCTCC

HpaI

CAATGTATTTGTGCTAAAATACAATGCCCTCAGTTCTTAACCAGGTAATCAGCAGTTGGCTGTCTAGCTGAAAACCTTGAGACCTTGTGTTAACCA
TTTTTTTTATTTAACATGATTGTTGAAGGAGAGAATTGACCTCCCAATGTAGGGCACTTTAGCACCCCCCCTCTCAGACAAATAGATATGGCCTTG
GCTTAAAGTTTTTTCTCTGCACTAATGTGGAGCCATAGAACCCTTGATAAAGCCAAGTCCCAAGTTTGTTTTCCCATCCTTACTTTAAAGGCCAAG

Exon 2

TAGGGTGACAAACAGCCTTTACCACCATTGCATCTGCCTTGCTGTGGGGATCAATAACAAATACCCTTTCCACTTTCAG TCT TGT TAT TTT
Ser Cys Tyr Phe

GAC CGG GAT GAT GTG GCC CTG AAG AAC TTT GCC AAA TAC TTT CTC CAT CAA TCT CAT GAA GAG AGG GAA CAT
Asp Arg Asp Asp Val Ala Leu Lys Asn Phe Ala Lys Tyr Phe Leu His Gln Ser His Glu Glu Arg Glu His
GCT GAG AAA CTG ATG AAG CTG CAG AAC CAG CGA GGT GGA CGA ATC TTC CTG CAG GAT ATC AAG GTAAGTAGACTA
Ala Glu Lys Leu Met Lys Leu Gln Asn Gln Arg Gly Gly Arg Ile Phe Leu Gln Asp Ile Lys
TGGGACTGCGTTAAATGAGCAGTNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNCTGCAGATGAATTGACATG

Exon 3

TTTCTTTGATTCAG AAA CCT GAC CGT GAT GAC TGG GAG AGC GGG CTG AAT GCA ATG AGG TGT GCA CTG CAC TTG
Lys Pro Asp Arg Asp Asp Trp Glu Ser Gly Leu Asn Ala Met Arg Cys Ala Leu His Leu
GAA AAG AGT GTG AAT CAG TCA CTA CTG GAA CTT CAC AAA CTG GCT ACT GAC AAG AAT GAT CCC CAC GTGAGTAT
Glu Lys Ser Val Asn Gln Ser Leu Leu Glu Leu His Lys Leu Ala Thr Asp Lys Asn Asp Pro His

Exon 4

CAGAAACACGGGGTGAGTGGAGATGATTTGCCACAGGGCTTGGGAGAGCTGACCAGTAACCCTGTCCCATGTTCTCTTTCCTAG TTA TGT GAC
Leu Cys Asp
TTC ATT GAG ACG CAT TAC CTG AAT GAG CAG GTG AAA TCC ATT AAA GAA CTG GGT GAC CAC GTG ACC AAC TTA
Phe Ile Glu Thr His Tyr Leu Asn Glu Gln Val Lys Ser Ile Lys Glu Leu Gly Asp His Val Thr Asn Leu
CGC AAG ATG GGA GCC CCT GAA TCT GGC ATG GCA GAA TAT CTC TTT GAC AAG CAC ACC CTG GGA CAC GGT GAT
Arg Lys Met Gly Ala Pro Glu Ser Gly Met Ala Glu Tyr Leu Phe Asp Lys His Thr Leu Gly His Gly Asp

AatII (5419)

GAG AGC TAA GCTGACGTCCCCAAGGCCATGTGACTTTACTGGTCACTGAGGCAGTGCATGCATGTCAGGCTGCCTTTATCTTTTCTATAAGTT
Glu Ser •••

Fer4

GCACCAAAACATCTGCTTAAAAGTTCTTTAATTTGTACCATTTCTTCAAATAAAGAATTTTGGTACCCAGCTCTTGTTGTGATTG

B.

Fer1

ATC TGT CCA TG GTGAGTGCGGCCTGGCCTTTGGCGGGGCGGAAAGAGGGTGCGGCCTGGCCTCCCTTGGGCCACTTGGTGAGCTGGCGGAGGG

TGGGTTGGGGCGTGGCCTGCTGCGGGCTTCCCCGCCTTCCAGCGCCCTTCTGGAAAATGGAGTTTGTCCGGGGTTCTTTCCAAAGGCAGGCAGCCCT
GCCGTGGCAAGTCTGAGCACCTAGCGCTTTGTGGCTCCTGCATAGACCAGGCACGTCATAACACCCGTGTTTTGAAGCCTTAGGGCTGTACAACTGT
CAGCCTCTCCAATCAACCCTGCAGTTAGGTGCATTTTCCTGCACTCTCGTCCCCTCCGGTCACATGGCCTGCAGGCTTCTCTGTTTGGGTGTACATC
CAGCTCCAGTTCCTCTGACTATGGCGGGTCTGCTTGGTCATGGTGTGGAATGGCAGCCCTGGGGCTTGGTACAAAGAGGCTTATCTCTTGTGAACTT
ACTCTAACCACTTCTGAAGCAGCGGCCTCTACATCTCTGCTTATCACAGAGCCTCACTTGCATTGAAACTTATCGCTAGGAATCTCCCCTTCTGTAA
TCACCCTGACCTTGCCAAGGCATCTAGAGTACTGTACGTTTTTAATTTTTATTTTGCACCAGTTGTTGCTTACTAACAGAAGTAGTAGGTAACATAC
TTGTTGGAAAAAGCCCACGGTTGGGAAAAAACCATTATCGTGGAATACAAATACACTGAGTGCCTAAAACTGAAAATCAAAGCTTCTCCCAATGTAT

HpaI

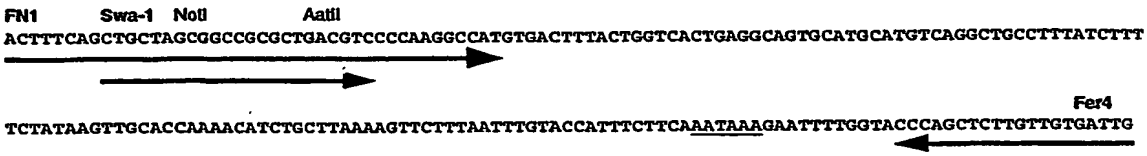
TTGTGCTAAAATACAATGCCCTCAGTTCTTAACCAGGTAATCAGCAGTTGGCTGTCTAGCTGAAAACCTTGAGACCTTGTGTTAACCATTTTTTTTA
TTTAACATGATTGTTGAAGGAGAGAATTGACCTCCCAATGTAGGGCACTTTAGCACCCCCCCTCTCAGACAAATAGATATGGCCTTGGCTTAAAGTT
TTTTCTCTGCACTAATGTGGAGCCATAGAACCCTTGATAAAGCCAAGTCCCAAGTTTGTTTTCCCATCCTTACTTTAAAGGCCAAGTAGGGTGACAA

Swa-2 NotI FN2

ACAGCCTTTACCACCATTGCATCTGCCTTGCTGTGGGGATCAATAACAAATACCCTTTCCACTTTCAGCTGCTAGCGGCCGCGCTGACGT

FIGURE 5A

C.


FN1 Swa-1 NotI AatII
ACTTTCAGCTGCTAGCGGCCGCGCTGACGTCCCCAAGGCCATGTGACTTTACTGGTCACTGAGGCAGTGCATGCATGTCAGGCTGCCTTTATCTTT
Fer4
TCTATAAGTTGCACCAAAACATCTGCTTAAAAGTTCTTTAATTTGTACCATTTCTTCAAATAAAGAATTTTGGTACCCAGCTCTTGTTGTGATTG

D.


Fer1
ATC TGT CCA TG GTGAGTGCGGCCTGGCCTTTGGCGGGGCGGAAAGAGGGTGCGGCCTGGCCTCCCTTGGGCCACTTGGTGAGCTGGCGGAGGG
TGGGTTGGGGCGTGGCCTGCTGCGGGCTTCCCCGCCTTCCAGCGCCCTTCTGGAAAATGGAGTTTGTCCGGGGTTCTTTCCAAAGGCAGGCAGCCCT
GCCGTGGCAAGTCTGAGCACCTAGCGCTTTGTGGCTCCTGCATAGACCAGGCACGTCATAACACCCGTGTTTTGAAGCCTTAGGGCTGTACAACTGT
CAGCCTCTCCAATCAACCCTGCAGTTAGGTGCATTTTCCTGCACTCTCGTCCCCTCCGGTCACATGGCCTGCAGGCTTCTCTGTTTGGGTGTACATC
CAGCTCCAGTTCCTCTGACTATGGCGGGTCTGCTTGGTCATGGTGTGGAATGGCAGCCCTGGGGCTTGGTACAAAGAGGCTTATCTCTTGTGAACTT
ACTCTAACCACTTCTGAAGCAGCGGCCTCTACATCTCTGCTTATCACAGAGCCTCACTTGCATTGAAACTTATCGCTAGGAATCTCCCCTTCTGTAA
TCACCCTGACCTTGCCAAGGCATCTAGAGTACTGTACGTTTTTAATTTTTATTTTGCACCAGTTGTTGCTTACTAACAGAAGTAGTAGGTAACATAC
TTGTTGGAAAAAGCCCACGGTTGGGAAAAAACCATTATCGTGGAATACAAATACACTGAGTGCCTAAAACTGAAAATCAAAGCTTCTCCCAATGTAT
HpaI
TTGTGCTAAAATACAATGCCCTCAGTTCTTAACCAGGTAATCAGCAGTTGGCTGTCTAGCTGAAAACCTTGAGACCTTGTGTTAACCATTTTTTTTA
TTTAACATGATTGTTGAAGGAGAGAATTGACCTCCCAATGTAGGGCACTTTAGCACCCCCCCTCTCAGACAAATAGATATGGCCTTGGCTTAAAGTT
TTTTCTCTGCACTAATGTGGAGCCATAGAACCCTTGATAAAGCCAAGTCCCAAGTTTGTTTTCCCATCCTTACTTTAAAGGCCAAGTAGGGTGACAA
Swa-1 SwaI Swa-2 NotI AatII
ACAGCCTTTACCACCATTGCATCTGCCTTGCTGTGGGGATCAATAACAAATACCCTTTCCATTTAAATCTGCTAGCGGCCGCTGACGTCCCCAAGGC
CATGTGACTTTACTGGTCACTGAGGCAGTGCATGCATGTCAGGCTGCCTTTATCTTTTCTATAAGTTGCACCAAAACATCTGCTTAAAAGTTCTTTA
Fer4
ATTTGTACCATTTCTTCAAATAAAGAATTTTGGTACCCAGCTCTTGTTGTGATTG

FIGURE 5B

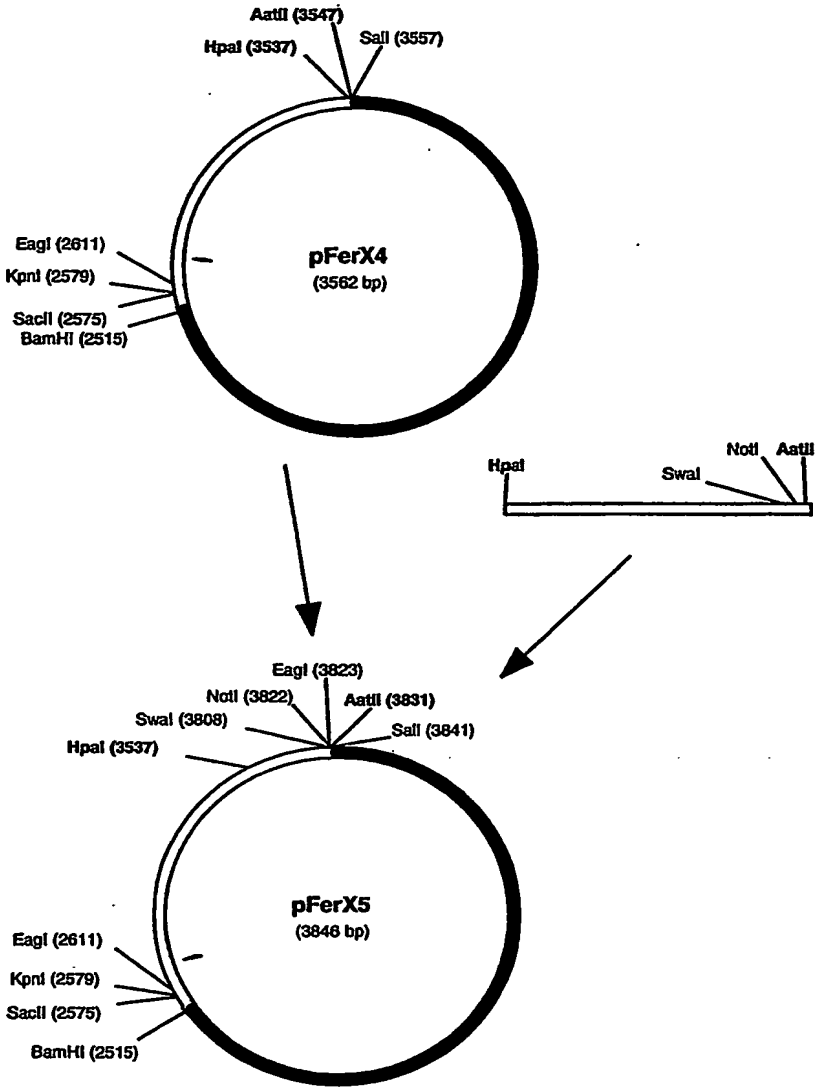

AatII (3547)
HpaI (3537)
SalI (3557)
EagI (2611)
KpnI (2579)
SacII (2575)
BamHI (2515)
pFerX4
(3562 bp)
HpaI
SwaI
NotI
AatII
EagI (3823)
NotI (3822)
AatII (3831)
SwaI (3808)
SalI (3841)
HpaI (3537)
pFerX5
(3846 bp)
EagI (2611)
KpnI (2579)
SacII (2575)
BamHI (2515)

FIGURE 6

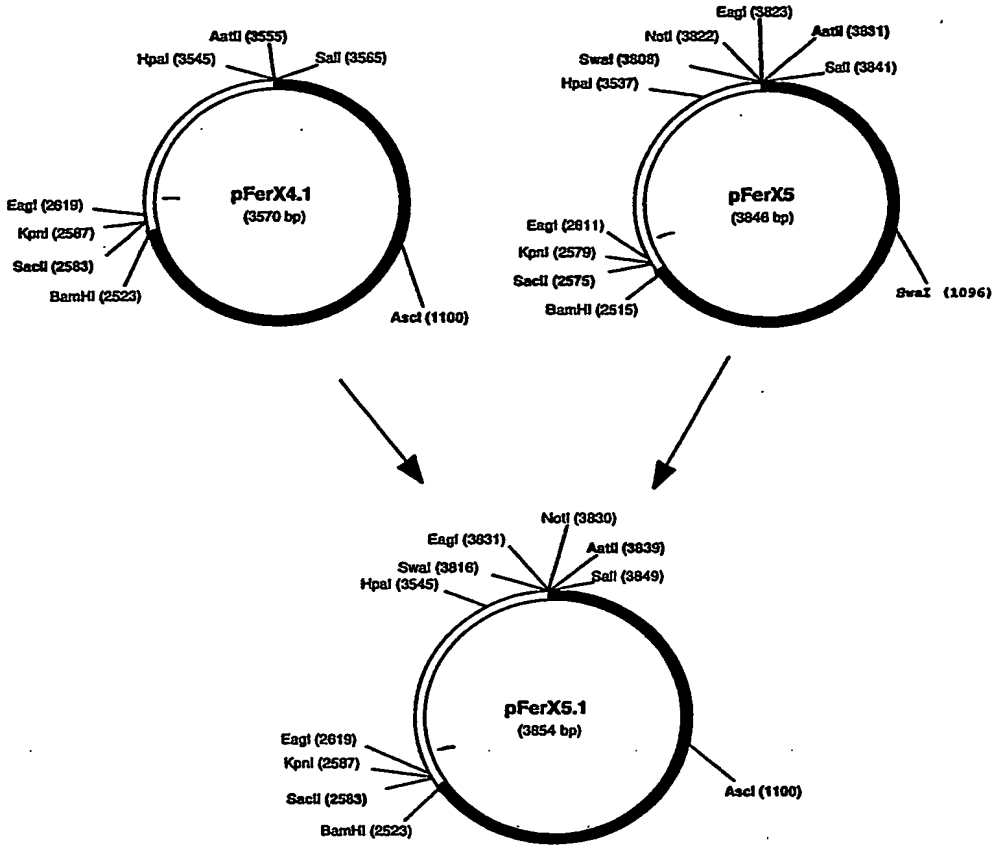

AatII (3555)
HpaI (3545)
SalI (3565)
pFerX4.1
(3570 bp)
EagI (2619)
KpnI (2587)
SacII (2583)
BamHI (2523)
AscI (1100)
EagI (3823)
NotI (3822)
AatII (3831)
SwaI (3808)
SalI (3841)
HpaI (3537)
pFerX5
(3846 bp)
EagI (2611)
KpnI (2579)
SacII (2575)
BamHI (2515)
SwaI (1096)
NotI (3830)
EagI (3831)
AatII (3839)
SwaI (3816)
SalI (3849)
HpaI (3545)
pFerX5.1
(3854 bp)
EagI (2619)
KpnI (2587)
SacII (2583)
BamHI (2523)
AscI (1100)

FIGURE 7

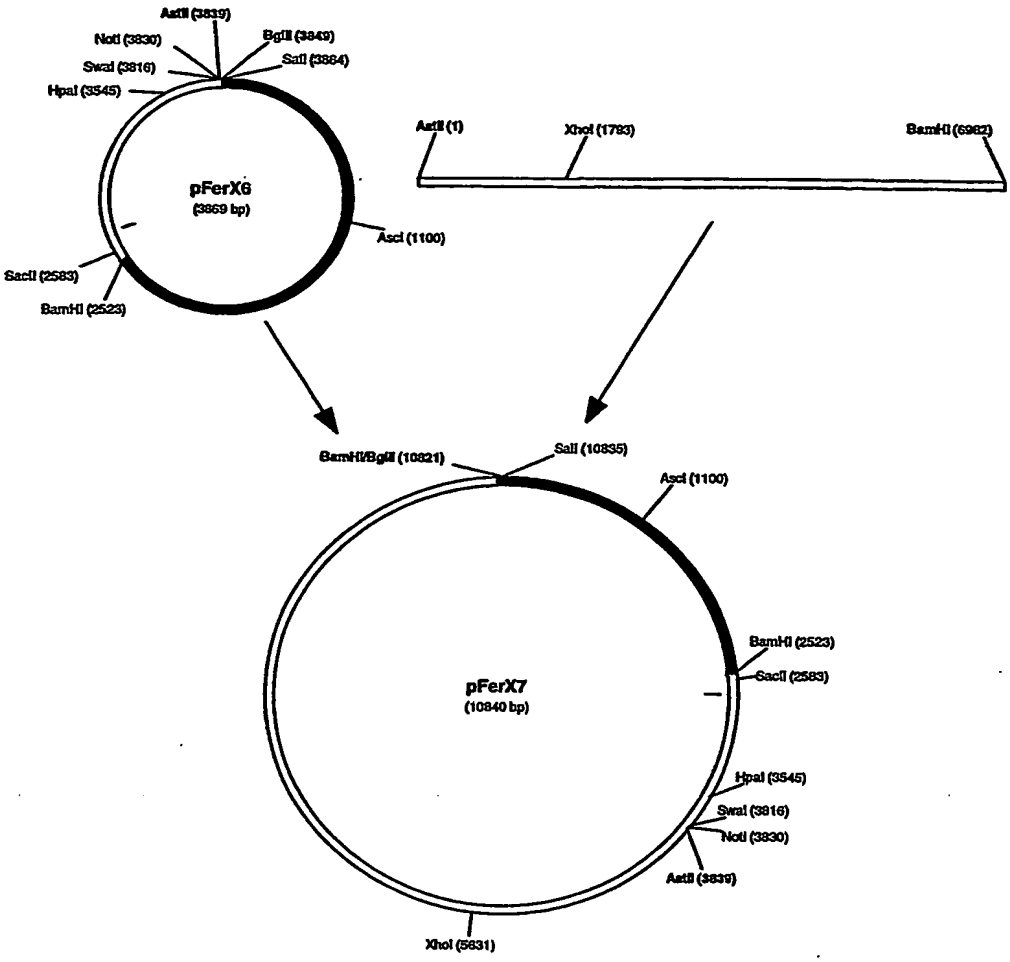

AatII (3839)
NotI (3830)
BglII (3849)
SalI (3864)
SwaI (3816)
HpaI (3545)
pFerX6
(3869 bp)
AscI (1100)
SacII (2583)
BamHI (2523)
AatII (1)
XhoI (1793)
BamHI (6962)
BamHI/BglII (10821)
SalI (10835)
AscI (1100)
BamHI (2523)
SacII (2583)
pFerX7
(10840 bp)
HpaI (3545)
SwaI (3816)
NotI (3830)
AatII (3839)
XhoI (5831)

FIGURE 8

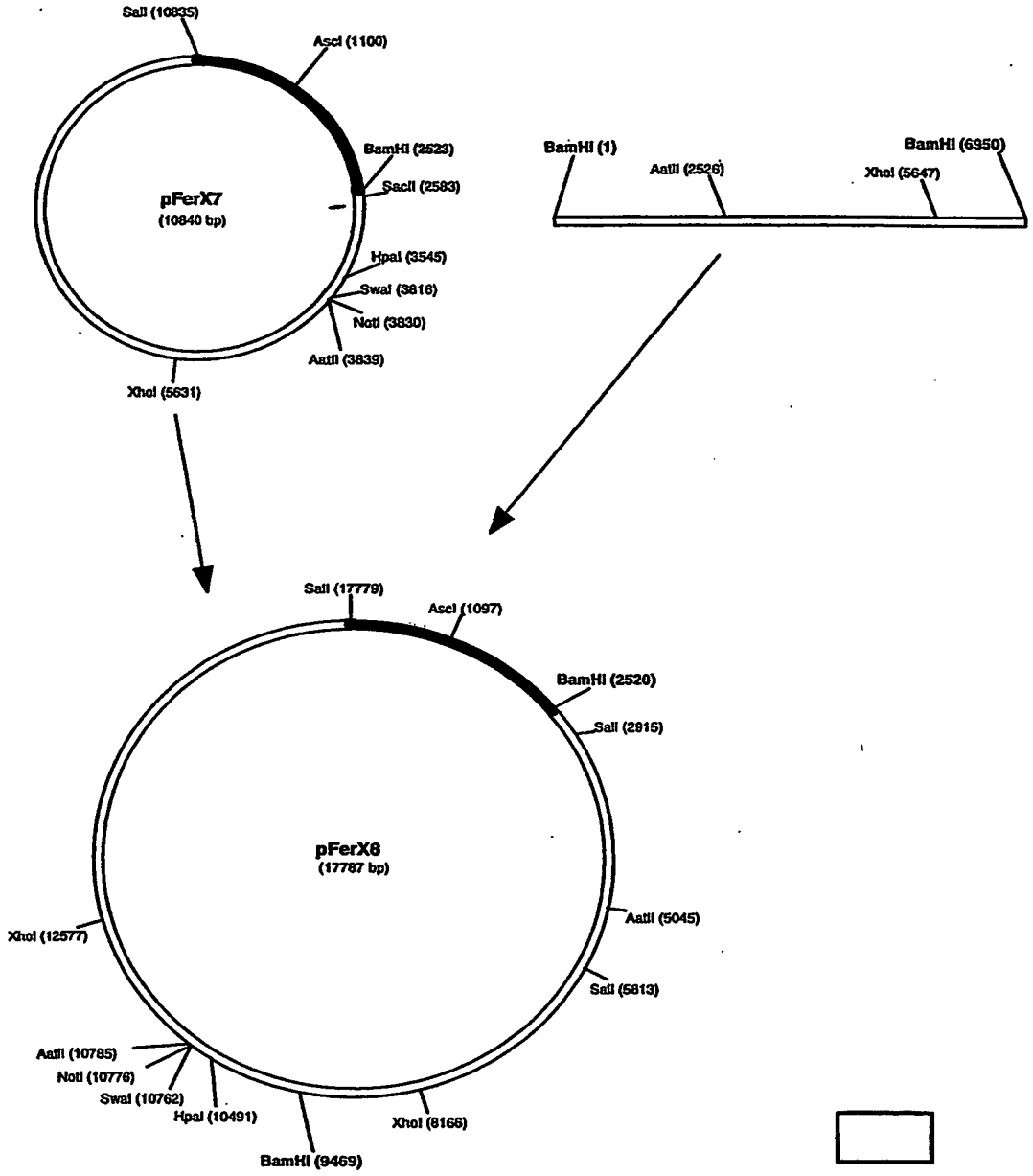

SalI (10835)
AscI (1100)
BamHI (2523)
SacII (2583)
pFerX7
(10840 bp)
HpaI (3545)
SwaI (3816)
NotI (3830)
AatII (3839)
XhoI (5631)
BamHI (1)
AatII (2526)
XhoI (5647)
BamHI (6950)
SalI (17779)
AscI (1097)
BamHI (2520)
SalI (2915)
pFerX8
(17787 bp)
AatII (5045)
SalI (5813)
XhoI (8166)
BamHI (9469)
HpaI (10491)
SwaI (10762)
NotI (10776)
AatII (10785)
XhoI (12577)

FIGURE 9

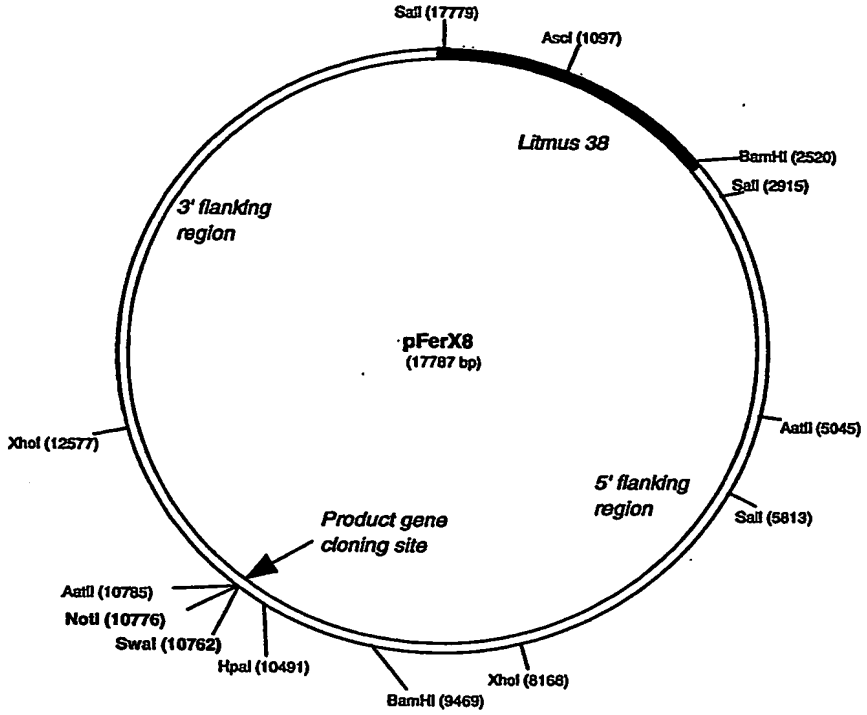

SalI (17779)
AscI (1097)
Litmus 38
BamHI (2520)
SalI (2915)
3' flanking region
pFerX8
(17787 bp)
AatII (5045)
XhoI (12577)
5' flanking region
SalI (5813)
Product gene cloning site
AatII (10785)
NotI (10776)
SwaI (10762)
HpaI (10491)
XhoI (8168)
BamHI (9469)

FIGURE 10

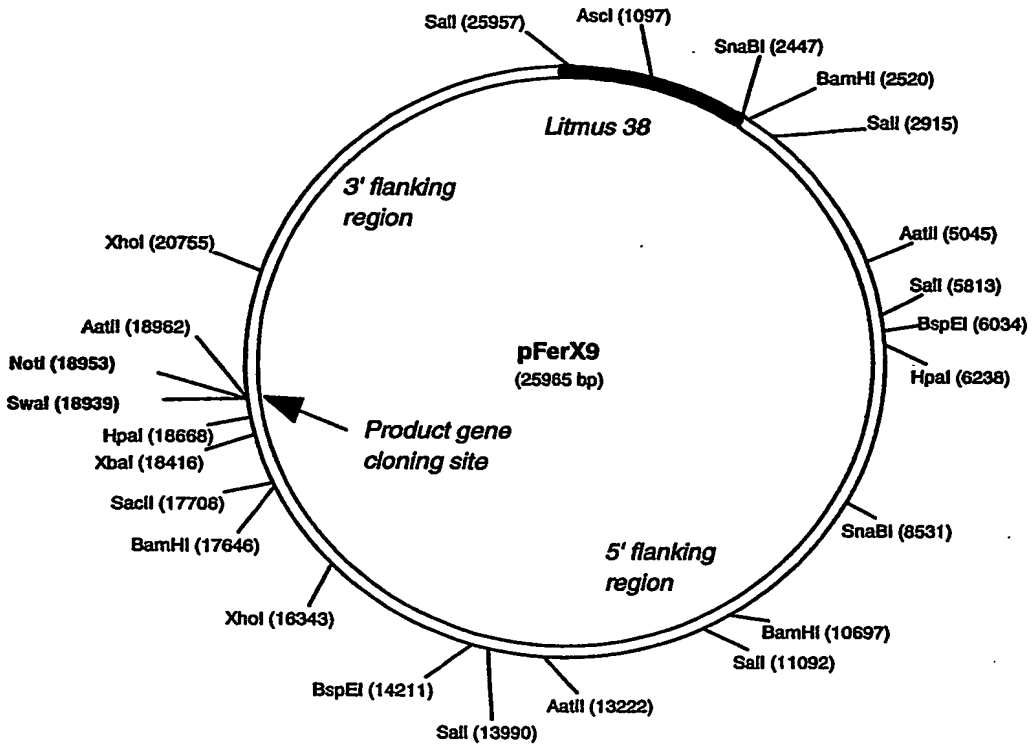

SalI (25957)
AscI (1097)
SnaBI (2447)
BamHI (2520)
SalI (2915)
Litmus 38
3' flanking region
AatII (5045)
SalI (5813)
BspEI (6034)
HpaI (6238)
SnaBI (8531)
5' flanking region
BamHI (10697)
SalI (11092)
AatII (13222)
SalI (13990)
BspEI (14211)
XhoI (16343)
BamHI (17646)
SacII (17708)
XbaI (18416)
HpaI (18668)
SwaI (18939)
NotI (18953)
AatII (18962)
XhoI (20755)
pFerX9
(25965 bp)
Product gene cloning site

FIGURE 11

SacII
BamHI (9469) CAP (9521) KpnI (9533)
GGATCCCGCTATAAAGTGCGGCCCGCTGGTCCCTACGCCAGACGTTCTCGCCCAGAGTCGCCGCGGTACCGGTGCTCG
ACCCCTCCGACCCCCGTCCGGCCGCTTTGAGCCTGAGCCCTTTGCAACTTCGTCGCTCCGCCGCTCCAGCGTCGCCTC
CGCGCCTCGCCCAGCCGCCATC ATG gtgagtgcggcctggcctttggcggggcggaaagagggtgcggcctggcct
▶Met
ccttgggccacttggtgagctggcggagggtgggttggggcgtggcctgctgcgggcttccccgccttccagcgccc
ttctggaaaatggagtttgtccggggttctttccaaaggcaggcagccctgccgtggcaagtctgagcacctagcgct
ttgtggctcctgcatagaccaggcacgtcataacacccgtgttttgaagccttagggctgtacaactgtcagcctctc
caatcaaccctgcagttaggtgcattttcctgcactctcgtcccctccggtcacatggcctgcaggcttctctgtttg
ggtgtacatccagctccagttcctctgactatggcgggtctgcttggtcatggtgtggaatggcagccctggggcttg
gtacaaagaggcttatctcttgtgaacttactctaaccacttctgaagcagcggcctctacatctctgcttatcacag
agcctcacttgcattgaaacttatcgctaggaatctccccttctgtaatcaccctgaccttgccaaggcatctagagt
actgtacgtttttaatttttatttgcaccagttgttgcttactaacagaagtagtaggtaacatacttgttggaaaa
agcccacggttgggaaaaaaccattatcgtggaatacaaatacactgagtgcctaaaactgaaaatcaaagcttctcc
caatgtatttgtgctaaaatacaatgccctcagttcttaaccaggtaatcagcagttggctgtctagctgaaaaccctt
gagaccttgtgttaaccatttttttttatttaacatgattgttgaaggagagaattgacctcccaatgtagggcacttt
agcaccccccctctcagacaaatagatatggccttggcttaaagtttttttctctgcactaatgtggagccatagaacc
cttgataaagccaagtcccaagtttgttttcccatccttactttaaaggccaagtagggtgacaaacagcctttacca
AatII (10785)
SwaI (10762) NotI (10776)
ccattgcatctgccttgctgtggggatcaataacaaataccctttccatttAAATCTGCTAGCGGCCGCTGACGTCCC
CAAGGCCATGTGACTTTACTGGTCACTGAGGCAGTGCATGCATGTCAGGCTGCCTTTATCTTTTCTATAAGTTGCACC
KpnI (10927)
AAAACATCTGCTTAAAAGTTCTTTAATTTGTACCATTTCTTCAAATAAAGAATTTTGGTACCCAGCTCTTGTTGTGAT
TGAGGATGAGCGCACCAGCTTCCCTTGCGTCGGCTATACTAACCACACTGCA

FIGURE 12

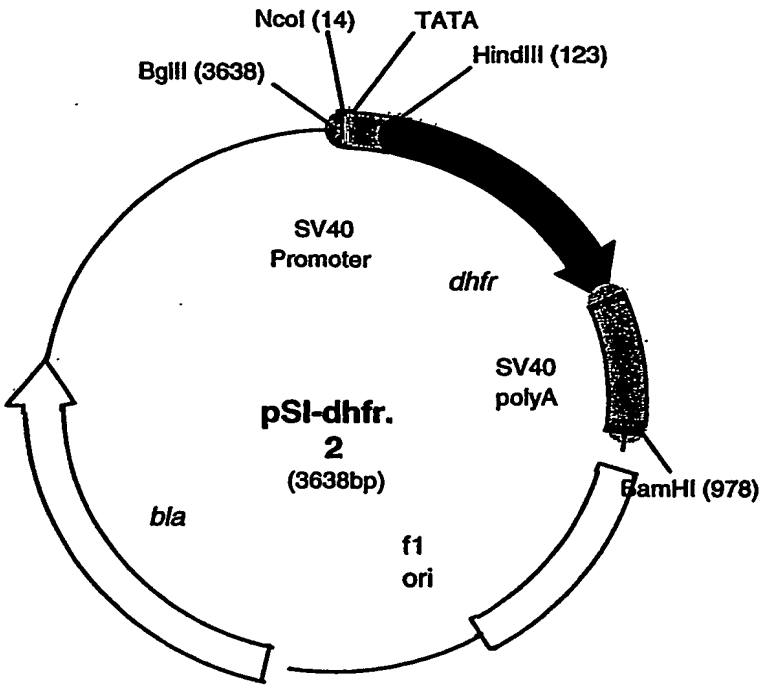

NcoI (14)
TATA
HindIII (123)
BglII (3638)
SV40
Promoter
dhfr
SV40
polyA
pSI-dhfr.
2
(3638bp)
BamHI (978)
bla
f1
ori

FIGURE 13

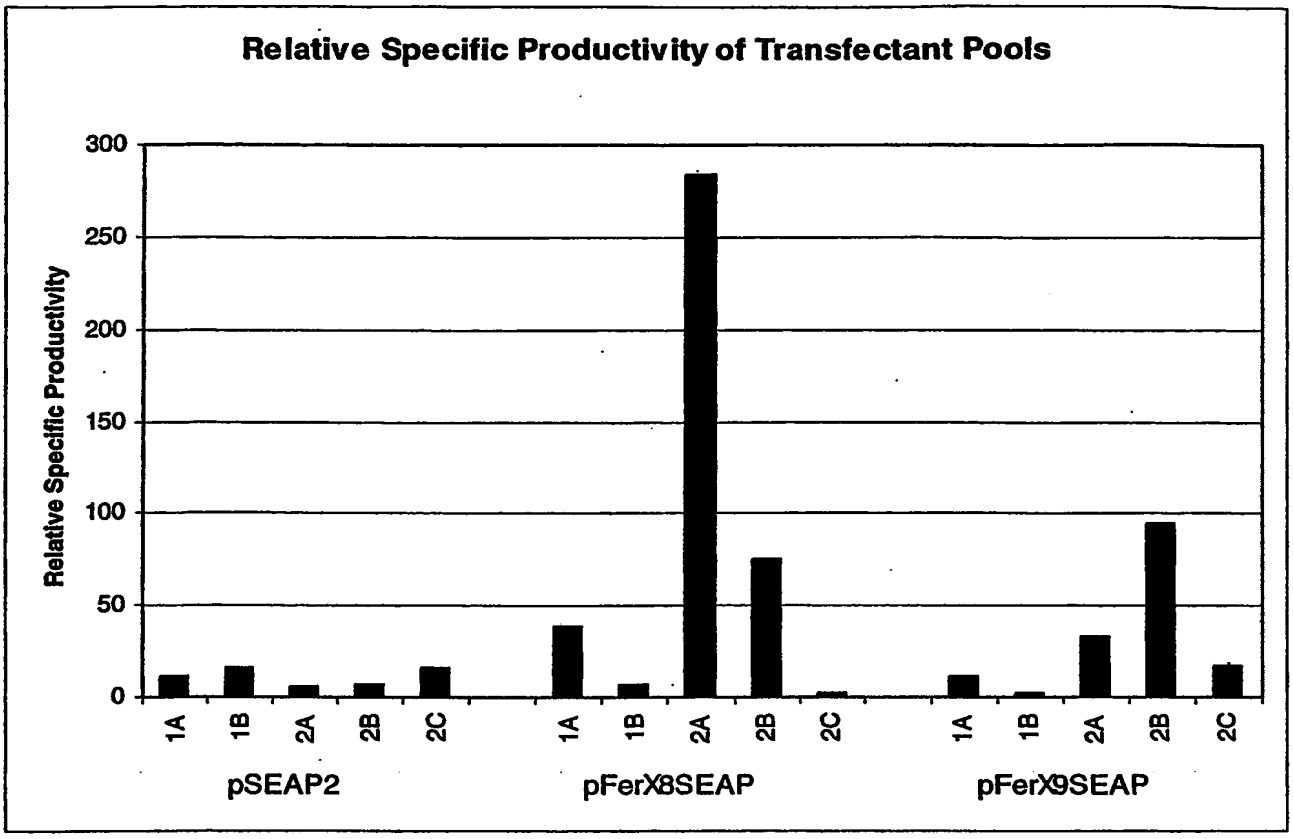

Relative Specific Productivity of Transfectant Pools
Relative Specific Productivity
300
250
200
150
100
50
0
1A
1B
2A
2B
2C
pSEAP2
pFerX8SEAP
pFerX9SEAP

FIGURE 14

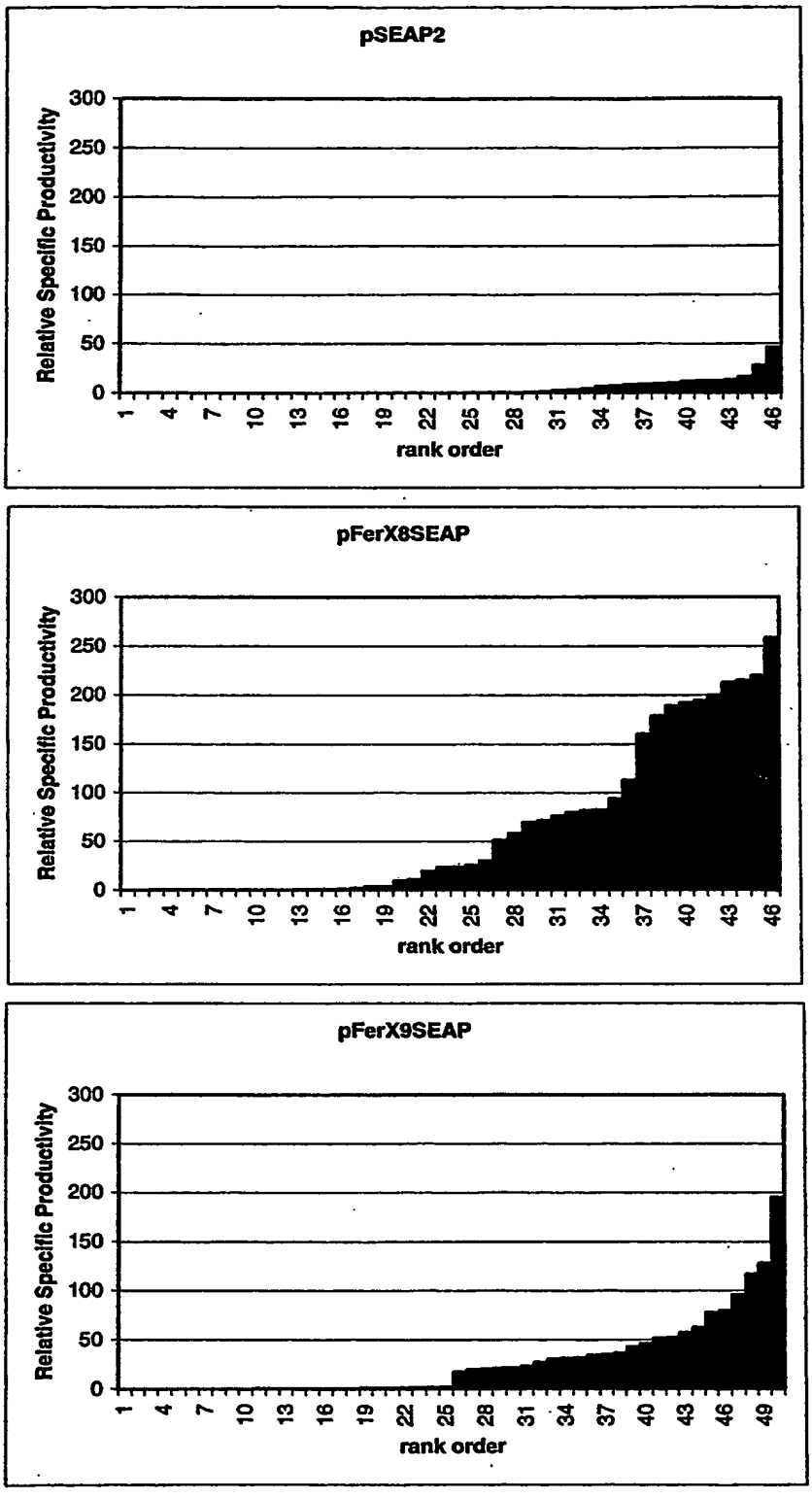

pSEAP2
Relative Specific Productivity
300
250
200
150
100
50
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46
rank order
pFerX8SEAP
Relative Specific Productivity
300
250
200
150
100
50
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46
rank order
pFerX9SEAP
Relative Specific Productivity
300
250
200
150
100
50
0
1 4 7 10 13 16 19 22 25 28 31 34 37 40 43 46 49
rank order

FIGURE 15